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(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) 5-Methanesulfonamido-6-(2-Pyridylthio)-1-Indanones as Inhibitors of Cyclooxygenase-2

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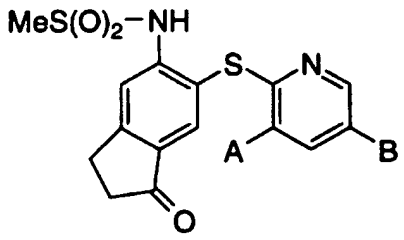
TITLE OF THE INVENTION

5-METHANESULFONAMIDO-6-(2-PYRIDYLTHIO)-1-INDANONES AS INHIBITORS OF CYCLOOXYGENASE-2

ABSTRACT OF THE DISCLOSURE

The invention encompasses the novel compound of Formula I as well as a method of treating cyclooxygenase-2 mediated diseases comprising administration to a patient in need of such treatment of a non-toxic therapeutically effective amount of a compound of Formula I.



I

The invention also encompasses certain pharmaceutical compositions for treatment of cyclooxygenase-2 mediated diseases comprising compounds of Formula I.

TITLE OF THE INVENTION

5-METHANESULFONAMIDO-6-(2-PYRIDYLTHIO)-1-INDANONES AS INHIBITORS OF CYCLOOXYGENASE-2

BACKGROUND OF THE INVENTION

This invention relates to methods of treating cyclooxygenase mediated diseases and certain pharmaceutical compositions therefor.

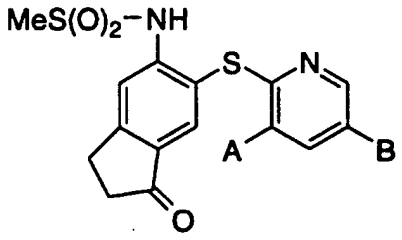
Non-steroidal, antiinflammatory drugs exert most of their antiinflammatory, analgesic and antipyretic activity and inhibit hormone-induced uterine contractions and certain types of cancer growth through inhibition of prostaglandin G/H synthase, also known as cyclooxygenase. Initially, only one form of cyclooxygenase was known, this corresponding to cyclooxygenase-1 or the constitutive enzyme, as originally identified in bovine seminal vesicles. More recently the gene for a second inducible form of cyclooxygenase (cyclooxygenase-2) has been cloned, sequenced and characterized initially from chicken, murine and human sources. This enzyme is distinct from the cyclooxygenase-1 which has been cloned, sequenced and characterized from various sources including the sheep, the mouse and man. The second form of cyclooxygenase, cyclooxygenase-2, is rapidly and readily inducible by a number of agents including mitogens, endotoxin, hormones, cytokines and growth factors. As prostaglandins have both physiological and pathological roles, we have concluded that the constitutive enzyme, cyclooxygenase-1, is responsible, in large part, for endogenous basal release of prostaglandins and hence is important in their physiological functions such as the maintenance of gastrointestinal integrity and renal blood flow. In contrast, we have concluded that the inducible form, cyclooxygenase-2, is mainly responsible for the pathological effects of prostaglandins where rapid induction of the enzyme would occur in response to such agents as inflammatory agents, hormones, growth factors, and cytokines. Thus, a selective inhibitor of cyclooxygenase-2 will have similar antiinflammatory, antipyretic and analgesic properties to a conventional non-steroidal antiinflammatory drug, and in addition would inhibit hormone-induced uterine contractions and have potential

anti-cancer effects, but will have a diminished ability to induce some of the mechanism-based side effects. In particular, such a compound should have a reduced potential for gastrointestinal toxicity, a reduced potential for renal side effects, a reduced effect on bleeding times and possibly a lessened ability to induce asthma attacks in aspirin-sensitive asthmatic subjects.

A brief description of the potential utility of cyclooxygenase-2 is given in an article by John Vane, *Nature*, Vol. *367*, pp. 215-216, 1994.

SUMMARY OF THE INVENTION

The invention encompasses the novel compound of Formula I as well as a method of treating cyclooxygenase-2 mediated diseases comprising administration to a patient in need of such treatment of a non-toxic therapeutically effective amount of a compound of Formula I.

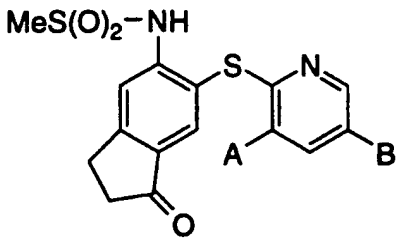


I

The invention also encompasses certain pharmaceutical compositions for treatment of cyclooxygenase-2 mediated diseases comprising compounds of Formula I.

DETAILED DESCRIPTION OF THE INVENTION

The invention encompasses the novel compound of Formula I as well as a method of treating cyclooxygenase-2 mediated diseases comprising administration to a patient in need of such treatment of a non-toxic therapeutically effective amount of a compound of Formula I.



I

wherein:

A and B are independently:

(a) hydrogen,
(b) F, Cl, Br, or I,
(c) methyl or ethyl,
(d) ethenyl or ethynyl,
(e) OCH_3 or OCF_3,
(f) SCH_3 or SCF_3,
(g) CN, or
(h) N_3.

Within this embodiment there is the genus of compounds of formula I wherein

A is

(a) hydrogen,
(b) F, Cl, Br,
(c) methyl or ethyl,
(d) ethenyl or ethynyl,
(e) OCH_3,
(f) SCH_3,
(g) CN;

B is

(a) hydrogen,
(b) F, Cl, Br,
(c) methyl,
(d) ethenyl or ethynyl,

(e) OCH_3,
(f) SCH_3, or
(g) CN

but A and B are not simultaneously hydrogen.

Within this genus there is the class of compound of formula I wherein

A is

(a) hydrogen,
(b) F, Cl, Br,
(c) methyl,

B is

(a) hydrogen,
(b) F, Cl, Br, or
(c) methyl.

but A and B are not simultaneously hydrogen.

The invention is illustrated by the compounds of Examples 1 through 17 as disclosed herein.

The pharmaceutical compositions of the present invention comprise a compound of Formula I as an active ingredient or a pharmaceutically acceptable salt, thereof, and may also contain a pharmaceutically acceptable carrier and optionally other therapeutic ingredients. The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases including inorganic bases and organic bases. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, zinc, and the like. Particularly preferred are the ammonium, calcium, magnesium, potassium, and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion exchange resins, such as arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine,

diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like.

It will be understood that in the discussion of methods of treatment which follows, references to the compounds of Formula I are meant to also include the pharmaceutically acceptable salts.

The Compound of Formula I is useful for the relief of pain, fever and inflammation of a variety of conditions including rheumatic fever, symptoms associated with influenza or other viral infections, common cold, low back and neck pain, dysmenorrhea, headache, toothache, sprains and strains, myositis, neuralgia, synovitis, arthritis, including rheumatoid arthritis, degenerative joint diseases (osteoarthritis), gout and ankylosing spondylitis, bursitis, burns, injuries, following surgical and dental procedures. In addition, such a compound may inhibit cellular neoplastic transformations and metastic tumor growth and hence can be used in the treatment of cancer. Compound I may also be of use in the treatment and/or prevention of cyclooxygenase-mediated proliferative disorders such as may occur in diabetic retinopathy and tumour angiogenesis.

Compound I will also inhibit prostanoid-induced smooth muscle contraction by preventing the synthesis of contractile prostanoids and hence may be of use in the treatment of dysmenorrhea, premature labor, asthma and eosinophil related disorders. It will also be of use in the treatment of Alzheimer's disease, and for the prevention of bone loss (treatment of osteoporosis).

By virtue of its high cyclooxygenase-2 (COX-2) activity and/or its specificity for cyclooxygenase-2 over cyclooxygenase-1 (COX-1), Compound I will prove useful as an alternative to conventional non-steroidal antiinflammatory drugs (NSAID'S) particularly where such non-steroidal antiinflammatory drugs may be contra-indicated such as in patients with peptic ulcers, gastritis, regional enteritis, ulcerative colitis,

diverticulitis or with a recurrent history of gastrointestinal lesions; GI bleeding, coagulation disorders including anemia such as hypoprothrombinemia, haemophilia or other bleeding problems; kidney disease; those prior to surgery or taking anticoagulants.

Similarly, Compound I, will be useful as a partial or complete substitute for conventional NSAID'S in preparations wherein they are presently co-administered with other agents or ingredients. Thus in further aspects, the invention encompasses pharmaceutical compositions for treating cyclooxygenase-2 mediated diseases as defined above comprising a non-toxic therapeutically effective amount of the compound of Formula I as defined above and one or more ingredients such as another pain reliever including acetominophen or phenacetin; a potentiator including caffeine; an H2-antagonist, aluminum or magnesium hydroxide, simethicone, a decongestant including phenylephrine, phenylpropanolamine, pseudophedrine, oxymetazoline, ephinephrine, naphazoline, xylometazoline, propylhexedrine, or levo-desoxyephedrine; an antiitussive including codeine, hydrocodone, caramiphen, carbetapentane, or dextramethorphan; a diuretic; a sedating or non-sedating antihistamine. In addition the invention encompasses a method of treating cyclooxygenase mediated diseases comprising: administration to a patient in need of such treatment a non-toxic therapeutically effect amount of the compound of Formula I, optionally co-administered with one or more of such ingredients as listed immediately above.

For the treatment of any of these cyclooxygenase mediated diseases Compound I may be administered orally, topically, parenterally, by inhalation spray or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In addition to the treatment of warm-blooded animals such as mice, rats, horses, cattle sheep, dogs, cats, etc., the compound of the invention is effective in the treatment of humans.

As indicated above, pharmaceutical compositions for treating cyclooxygenase-2 mediated diseases as defined may optionally include one or more ingredients as listed above.

The pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example, magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated by the technique described in the U.S. Patent 4,256,108; 4,166,452; and 4,265,874 to form osmotic therapeutic tablets for control release.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredients is mixed with water or miscible solvents such as propylene glycol, PEGs and ethanol, or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions contain the active material in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example, sodium carboxymethylcellulose, methylcellulose, hydroxy-propylmethycellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl, p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose, saccharin or aspartame.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example, arachis oil, olive oil, sesame oil or coconut oil, or in mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example, beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example, sweetening, flavoring and coloring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of an oil-in-water emulsions. The oily phase may be a vegetable oil, for example, olive oil or arachis oil, or a mineral oil, for example, liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring phosphatides, for example, soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example, sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example, polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavouring agents.

Syrups and elixirs may be formulated with sweetening agents, for example, glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. Cosolvents such as ethanol, propylene glycol or polyethylene glycols may also be used. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Compound I may also be administered in the form of a suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

For topical use, creams, ointments, gels, solutions or suspensions, etc., containing the compound of Formula I are employed. (For purposes of this application, topical application shall include mouth washes and gargles.) Topical formulations may generally be comprised of a pharmaceutical carrier, cosolvent, emulsifier, penetration enhancer, preservative system, and emollient.

Dosage levels of the order of from about 0.01 mg to about 140 mg/kg of body weight per day are useful in the treatment of the above-indicated conditions, or alternatively about 0.5 mg to about 7 g per patient per day. For example, inflammation may be effectively treated by the administration of from about 0.01 to 50 mg of the compound per kilogram of body weight per day, or alternatively about 0.5 mg to about 3.5 g per patient per day.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a formulation intended for the oral administration of humans may contain from 0.5 mg to 5 g of active agent compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95 percent of the total composition. Dosage unit forms will generally contain between from about 1 mg to about 500 mg of an active ingredient, typically 25 mg, 50 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 800 mg, or 1000 mg.

It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

The compounds of the present invention can be prepared according to the following methods.

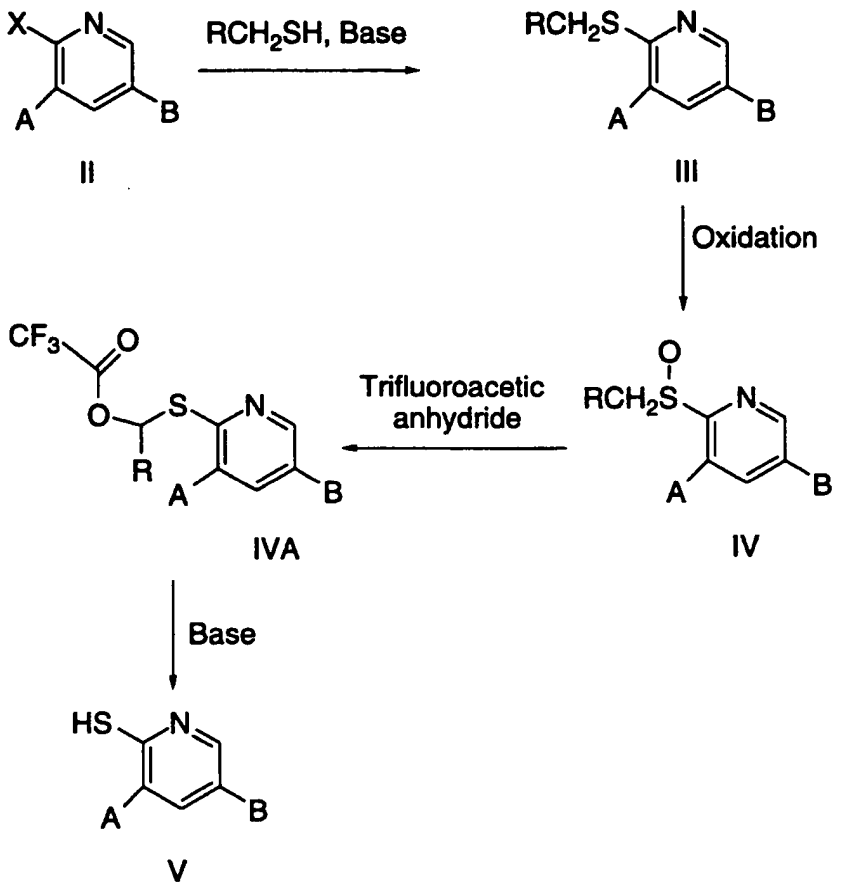
Preparation of 2-Mercaptopyridines

The 2-mercaptopyridines can be prepared according to the method of Phillips and Shapiro (*J. Chem. Soc., 584* (1942)) or method A as described below.

Method A

An appropriately substituted 2-halopyridine II (X = Cl or Br) is reacted with an alkylthiol under basic conditions to give sulfide III. Oxidation with one equivalent of an oxidizing agent such as MCPBA yields sulfoxide IV. Treatment with trifluoroacetic anhydride brings about the Pummerer rearrangement of IV to intermediate IVa. Upon basic hydrolysis IVa gives the 2-mercaptopyridine V.

METHOD A



Preparation of 5-Methanesulfonamido-6-(2-pyridylthio)-1-indanones

Method B

5-Aminoindane VI is acetylated, followed by bromination to give the 5-acetylamino-6-bromoindane VII. Oxidation with chromium trioxide in aqueous acetic acid, followed by acidic hydrolysis gives the 5-amino-6-bromo-1-indanone VIII. The amino group is converted to the nitro group by diazotization followed by treatment of the corresponding diazonium salt with sodium nitrite in the presence of copper powder. Subsequent protection of the carbonyl as a dioxolane provides 5-nitro-6-bromo-1-indanone ethylene ketal IX. Coupling with the appropriate 2-

mercaptopyridine V proceeds under basic conditions with or without the presence of a copper salt. Reduction of the nitro group with iron powder or tin(II) chloride in aqueous ethanol with concomitant hydrolysis of the ketal group leads to amino indanone X. Sulfonylation with excess methanesulfonyl chloride in the presence of triethylamine yields the corresponding bissulfonamide, which upon subsequent hydrolysis with sodium hydroxide yields the title Compound I.

METHOD B

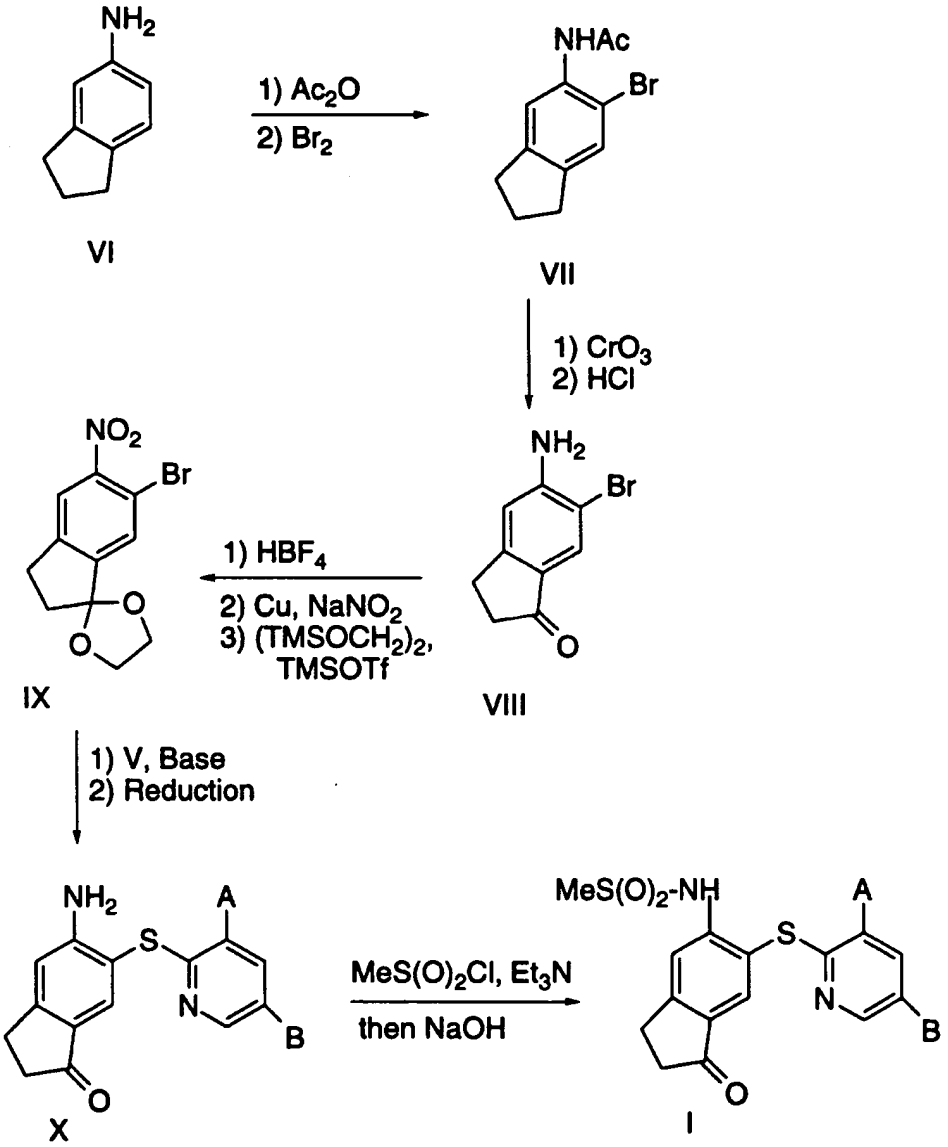

NH2
1) Ac2O
2) Br2
NHAc
Br
VI
VII
1) CrO3
2) HCl
NO2
Br
NH2
Br
1) HBF4
2) Cu, NaNO2
3) (TMSOCH2)2,
TMSOTf
O
O
O
IX
VIII
1) V, Base
2) Reduction
NH2
A
S
N
B
O
X
MeS(O)2-NH
MeS(O)2Cl, Et3N
then NaOH
I

Table I illustrates compounds of Formula I, which are representative of the present invention.

TABLE 1

NMR DATA FOR EXAMPLES

Example	A	B	NMR δ (ppm)
1	H	Cl	($CDCl_3$) 8.38 (s, 1H), 8.30 (s, 1H), 8.02 (s, 1H), 7.85 (s, 1H), 7.55 (d, 1H), 7.12 (d, 1H), 3.18 (t, 2H), 3.06 (s, 3H), 2.72 (t, 2H)
2	H	Me	($CDCl_3$) 9.24 (s, 1H), 8.20 (s, 1H), 8.05 (s, 1H), 7.85 (s, 1H), 7.38 (d, 1H), 7.14 (d, 1H), 3.16 (t, 2H), 3.02 (s, 3H), 2.70 (t, 2H), 2.25 (s, 3H)
3	H	Br	($CDCl_3$) 8.39 (s, 1H), 8.42 (s, 1H), 8.02 (s, 1H), 7.85 (s, 1H), 7.68 (d, 1H), 7.03 (d, 1H), 3.18 (t, 2H), 3.05 (s, 3H), 2.72 (t, 2H)

TABLE 1 (CONT'D)

Example	A	B	NMR δ (ppm)
4	Cl	H	($CDCl_3$) 8.12 (d, 1H), 8.00 (s, 1H), 7.88 (overlapping s, 2H), 7.66 (d, 1H), 7.04 (dd, 1H), 3.20 (t, 2H), 3.02 (s, 3H), 2.74 (t, 2H)
5	Cl	Cl	($CDCl_3$), 8.10 (s, 1H), 7.98 (s, 1H), 7.86 (s, 1H), 7.72 (brs, 1H), 7.68 (s, 1H), 3.20 (t, 2H), 3.05 (s, 3H), 2.72 (t, 3H)
6	H	H	($CDCl_3$) 8.90 (brs, 1H), 8.37 (m, 1H), 8.08 (s, 1H), 7.39 (s, 1H), 7.59 (m, 1H), 7.22 (m, 1H), 7.12 (m, 1H), 3.21 (m, 2H), 3.05 (s, 3H), 2.73 (m, 2H)
7	F	H	
8	H	F	
9	F	F	
10	F	Cl	
11	F	Br	
12	Cl	F	
13	H	OCH_3	
14	H	SCH_3	
15	H	$CH=CH_2$	
16	H	$C\equiv CH$	
17	H	CN	

Table 2

Biological Data for Representative Examples

Example #	COX-2 I(50 (nm)	COX-1 I (50(μM)
1	<100	>10
2	~40	>10
3	94% @ 100 nM	>10
4	63% 100 nM	>10
5	100 % @ 100 nM	>10

Assays for determining Biological Activity

The compound of Formula I can be tested using the following assays to determine their cyclooxygenase-2 inhibiting activity.

Inhibition of Cyclooxygenase Activity

Compounds were tested as inhibitors of cyclooxygenase activity in whole cell cyclooxygenase assays. Both of these assays measured prostaglandin E_2 synthesis in response to arachidonic acid, using a radioimmunoassay. Cells used for these assays were human osteosarcoma 143 cells (which specifically express cyclooxygenase-2) and human U-937 cells (which specifically express cyclooxygenase-1). In these assays, 100% activity is defined as the difference between prostaglandin E_2 synthesis in the absence and presence of arachidonate addition.

Assay

For cyclooxygenase assays, osteosarcoma cells are cultured in 1 mL of media in 24-well multidishes (Nunclon) until confluent (1-2 x 10^5 cells/well). U-937 cells are grown in spinner flasks and resuspended to a final density of 1.5 x 10^6 cells/mL in 24-well multidishes (Nunclon). Following washing and resuspension of osteosarcoma and U-937 cells in 1 mL of HBSS, 1 μL of a DMSO solution of test compound or DMSO

vehicle is added, and samples gently mixed. All assays are performed in triplicate. Samples are then incubated for 5 or 15 minutes at 37°C, prior to the addition of arachidonic acid. Arachidonic acid (peroxide-free, Cayman Chemical) is prepared as a 10 mM stock solution in ethanol and further diluted 10-fold in HBSS. An aliquot of 10 µL of this diluted solution is added to the cells to give a final arachidonic acid concentration of 10 µM. Control samples are incubated with ethanol vehicle instead of arachidonic acid. Samples are again gently mixed and incubated for a further 10 min at 37°C. For osteosarcoma cells, reactions are then stopped by the addition of 100 µL of 1N HCl with mixing and by the rapid removal of the solution from cell monolayers. For U-937 cells, reactions are stopped by the addition of 100 µL of 1N HCl with mixing. Samples are then neutralized by the addition of 100 µL of 1N NaOH and PGE_2 levels measured by radioimmunoassay.

Rat Paw Edema Assay - Protocol

Male Sprague-Dawley rats (150 - 200 g) were fasted overnight and were given po either vehicle (1% methocel or 5% Tween 80) or a test compound. One hr later, a line was drawn using a permanent marker at the level above the ankle in one hind paw to define the area of the paw to be monitored. The paw volume (V_0) was measured using a plethysmometer (Ugo-Basile, Italy) based on the principle of water displacement. The animals were then injected subplantarly with 50 µl of 1% carrageenan solution in saline (FMC Corp, Maine) into the paw using an insulin syringe with a 25-gauge needle (i.e. 500 µg carrageenan per paw). Three hr later, the paw volume (V_3) was measured and the increases in paw volume ($V_3 - V_0$) were calculated. The animals were sacrificed by CO_2 aphyxiation and the absence or presence of stomach lesions scored. Data were compared with the vehicle-control values and percent inhibition calculated. Since a maximum of 60-70% inhibition (paw edema) was obtained with standard NSAIDs, ED_{30} values were used for comparison. All treatment groups were coded to eliminate observer bias.

NSAID-Induced Gastrophathy in Rats

Rationale

The major side effect of conventional NSAIDs is their ability to produce gastric lesions in man. This action is believed to be caused by inhibition of COX-1 in the gastrointestinal tract. Rats are particularly sensitive to the actions of NSAIDS. In fact, rat models have been used commonly in the past to evaluate the gastrointestinal side effects of current conventional NSAIDs. In the present assay, NSAID-induced gastrointestinal damage is observed by measuring fecal ^{51}Cr excretion after systemic injection of ^{51}Cr-labeled red blood cells. Fecal ^{51}Cr excretion is a well-established and sensitive technique to detect gastrointestinal integrity in animals and man.

Methods

Male Sprague Dawley rats (150 - 200 g) are administered orally a test compound either once (acute dosing) or b.i.d. for 5 days (chronic dosing). Immediately after the administration of the last dose, the rats ate injected via a tail vein with 0.5 mL of ^{51}Cr-labeled red blood cells from a donor rat. The animals are placed individually in metabolism cages with food and water ad *lib*. Feces are collected for a 48 h period and ^{51}Cr fecal excretion is calculated as a percent of total injected dose.

^{51}Cr-labeled red blood cells are prepared using the following procedures. Ten mL of blood is collected in heparinized tubes via the vena cava from a donor rat. Plasma is removed by centrifugation and replenished with equal volume of HBSS. The red blood cells are incubated with 400 μCi of sodium ^{51}chromate for 30 min at 37°C. At the end of the incubation, the red blood cells are washed twice with 20 mL HBSS to remove free sodium ^{51}chromate. The red blood cells are finally reconstituted in 10 mL HBSS and 0.5 mL of the solution (about 20 μCi) is injected per rat.

Protein-Losing Gastropathy in Squirrel Monkeys

Rationale

Protein-losing gastropathy (manifested as appearance of cirulating cells and plasma proteins in the GI tract) is a significant and dose-limiting adverse response to standard NSAIDs. This can be quantitatively assessed by intravenous administration of $^{51}CrCl_3$ solution. This isotopic ion can avidly bind to cell and serum globins and cell endoplasmic reticulum. Measurement of radioactivity appearing in feces collected for 24 h after administration of the isotope thus provides a sensitive and quantitative index of protein-losing gastropathy.

Methods

Groups of male squirrel monkeys (0.8 to 1.4 kg) are treated by gavage with either 1% methocel or 5% Tween 80 in H_2O vehicles, (3

mL/kg b.i.d.) or test compounds at doses from 1 - 100 mg/kg b.i.d. for 5 days. Intravenous ^{51}Cr (5 μCi/kg in 1 ml/kg PBS) is administered 1 h after the last drug/vehicle dose, and feces collected for 24 h in a metabolism cage and assessed for excreted ^{51}Cr by gamma-counting. Venous blood is sampled 1 h and 8 h after the last drug dose, and plasma concentrations of drug measured by RP-HPLC.

The following abbreviations have the indicated meanings

Ac	=	acetyl
Bn	=	benzyl
DMAP	=	4-(dimethylamino)pyridine
DMF	=	N,N-dimethylformamide
DMSO	=	dimethyl sulfoxide
Et3N	=	triethylamine
HBSS	=	Hank's balanced salt solution
MCPBA	=	meta-chloroperbenzoic acid
Ms	=	methanesulfonyl = mesyl
NSAID	=	non-steroidal anti-inflammatory drug
PBS	=	phosphate buffered saline
Ph	=	phenyl
RP-HPLC	=	reverse phase high performance liquid chromatography.
r.t.	=	room temperature
THF	=	tetrahydrofuran
TLC	=	thin layer chromatography
TFAA	=	trifluoroacetic anhydride

The invention will now be illustrated by the following non-limiting examples in which, unless stated otherwise:

(i) all operations were carried out at room or ambient temperature, that is, at a temperature in the range 18-25°C;

(ii) evaporation of solvent was carried out using a rotary evaporator under reduced pressure (600-4000 pascals: 4.5-30 mm Hg) with a bath temperature of up to 60°C;

(iii) he course of reactions was followed by thin layer chromatography (TLC) and reaction times are given for illustration only; (iv) melting points are uncorrected and `d' indicates decomposition; the melting points given are those obtained for the materials prepared as described; polymorphism may result in isolation of materials with different melting points in some preparations;

(v) the structure and purity of all final products were assured by at least one of the following techniques: TLC, mass spectrometry, nuclear magnetic resonance (NMR) spectrometry or microanalytical data;

(vi) yields are given for illustration only;

(vii) when given, NMR data is in the form of delta (δ) values for major diagnostic protons, given in parts per million (ppm) relative to tetramethylsilane (TMS) as internal standard, determined at 300 MHz or 400 MHz using the indicated solvent; conventional abbreviations used for signal shape are: s. singlet; d. doublet; t. triplet; m. multiplet; br. broad; etc.: in addition "Ar" signifies an aromatic signal;

(viii) chemical symbols have their usual meanings; the following abbreviations have also been used v (volume), w (weight), b.p. (boiling point), m.p. (melting point), L (liter(s)), mL (milliliters), g (gram(s)), mg (milligrams(s)), mol (moles), mmol (millimoles), eq (equivalent(s)).

EXAMPLE 1

5-Methanesulfonamido-6-(5-chloro-2-pyridylthio)-1-indanone

Step 1: 5-Acetylaminoindane

To a solution of 5-aminoindane (10.0 g, 7.5 mmol) in CH_2Cl_2 (100 mL) was added dropwise Ac_2O (9.2 g, 9.0 mmol) over a period of 15 min. After further stirring for 30 min, the mixture was quenched with 1 M aqueous NaOH (100 mL). The CH_2Cl_2 layer was separated, washed successively with 1M aqueous HCl, brine, and was then dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Chromatography over silica gel, eluting with EtOAc:hexanes (1:1) afforded 12.2 g (85%) of the title compound as a light brown powder. ^1H NMR ($CDCl_3$): δ 7.44 (1H, s), 7.12 (3H, three overlapping s), 2.88 (4H, m), 2.15 (3H, s), 2.06 (2H, m).

Step 2: 5-Acetylamino-6-bromoindane

To a solution of 5-acetylaminoindane from Step 1, (53.0 g, 0.30 mol) in HOAc (1L) at 10°C, Br_2 (19.0 mL, 0.37 mol) was added dropwise over a period of 1 h. The mixture was further stirred at 10°C for 15 min, and was then diluted with H_2O until no more precipitate formed. The precipitate was collected, washed with H_2O and dried under vacuum to give 61 g (80%) of the title compound. ^1H NMR ($CDCl_3$): δ 8.14 (1H, s), 7.50 (1H, s), 7.38 (1H, s), 2.88 (4H, m), 2.20 (3H, s), 2.08 (2H, m).

Step 3: 5-Acetylamino-6-bromo-1-indanone

To a solution of 5-acetylamino-6-bromoindane from Step 2, (43.0 g, 0.17 mol) in HOAc (400 mL) at 50-55°C was added dropwise a solution of CrO_3 (70.0 g, 0.7 mol) in 50% aqueous HOAc (400 mL) over a period of 30 min. After further stirring for 15 min, the mixture was cooled to 0°C and quenched with 2-propanol (100 mL). Solvent was removed *in vacuo*, the residue was diluted with H_2O (1L) and extracted with EtOAc (2 x 500 mL). The combined EtOAc layer was washed with

0.5 M aqueous NaOH (1 L) and brine, dried over anhydrous $MgSO_4$ and concentrated to give 36 g (80%) of the title compound as a light brown solid which was contaminated with about 10% of 5-bromo-6-acetylamino-1-indanone.

^1H NMR ($CDCl_3$): δ 8.60 (1H, s), 7.98 (1H, s), 7.90 (1H, s), 3.10 (2H, t), 2.70 (2H, t), 2.30 (3H, s).

Step 4: 5-Amino-6-bromo-1-indanone

A mixture of 5-acetylamino-6-bromo-1-indanone from Step 3, (36.0 g, 0.13 mol) and 6 M aqueous HCl (800 mL) was refluxed for 1 h. The homogenous solution was then cooled to 0°C and adjusted to pH 8 with 10 M aqueous NaOH (~480 mL). The precipitate formed was collected, washed with H_2O and dried under vacuum to afford 30.0 g (quantitative) of the title compound as a light brown powder.

^1H NMR (acetone-d_6): δ 7.65 (1H, s), 6.90 (1H, s), 5.80 (2H, br s), 2.95 (2H, t), 2.50 (2H, t).

Step 5: 5-Nitro-6-bromo-1-indanone

To a suspension of 5-amino-6-bromo-1-indanone from Step 4, (30.0 g, 0.13 mol) in 20% aqueous HBF_4 (120 mL) at 0°C was added dropwise 4 M aqueous $NaNO_2$ (50 mL, 0.20 mol) over a period of 30 min. The mixture was stirred for 30 min after completion of addition. The resulting foamy suspension was added portionwise to a vigorously stirred mixture of Cu powder (40 g, 0.62 mol) and $NaNO_2$ (120 g, 1.74 mol) in H_2O (240 mL) at r.t. over a period of 15 min. During the addition, excessive foaming was broken up by the addition of small amounts of Et_2O. After further stirring for 30 min, the mixture was filtered through celite, and washed with EtOAc (5 x 300 mL). The EtOAc layer was separated, washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Chromatography over silica gel, eluting with CH_2Cl_2, yielded 17.5 g (51%) of the title compound as a pale yellow solid.

^1H NMR ($CDCl_3$): δ 8.10 (1H, s), 7.85 (1H, s), 3.20 (2H, t), 2.85 (2H, t); mass spectrum (DCl, CH_4) m/e 256 (M^++H).

Step 6: 5-Nitro-6-bromo-1-indanone ethylene ketal

To a suspension of 5-nitro-6-bromo-1-indanone from Step 5, (11.0 g, 43 mmol) and 1,2-bis(trimethylsilyloxy)ethane (22.0 mL, 90 mmol) in CH_2Cl_2 (90 mL) at r.t. was added trimethylsilyl trifluoromethanesulfonate (100 µL). The mixture was stirred for 2 h and the homogeneous solution was quenched with saturated aqueous $NaHCO_3$ (100 mL). The CH_2Cl_2 layer was separated, washed with brine, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Chromatography over silica gel, eluting with EtOAc:hexanes (2:5), furnished 10.2 g (79%) of the title compound as a pale yellow solid.
^1H NMR ($CDCl_3$): δ 7.70 (1H, s), 7.68 (1H, s), 4.15 (4H, m), 2.98 (2H, t), 2.38 (2H, t).

Step 7: 5-Nitro-6-(5-chloro-2-pyridylthio)-1-indanone ethylene ketal

To a mixture of 5-nitro-6-bromo-1-indanone ethylene ketal from Step 6, (850 mg, 2.83 mmol) and 5-chloro-2-mercaptopyridine (350 mg, 2.40 mmol) in DMF (10 mL) was added powdered KOH (140 mg, 2.5 mmol) at r.t.. The resulting mixture was then heated at 120°C for 2 h. After cooling to r.t., the mixture was diluted with H_2O and extracted with EtOAc (2x). The combined EtOAc extracts were washed with dilute aqueous NaCl solution (3x), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Chromatography over silica gel and elution with hexanes:EtOAc (2:1) afforded the title compound (800 mg, 77%) as a pale yellow solid.
^1H NMR ($CDCl_3$): δ 8.42 (s, 1H), 7.88 (s, 1H), 7.58 (d, 1H), 7.42 (s, 1H), 7.20 (d, 1H), 4.05 (s, 4H), 3.00 (t, 2H), 2.36 (t, 2H).

Step 8: 5-Amino-6-(5-chloro-2-pyridylthio)-1-indanone

A mixture of 5-nitro-6-(5-chloro-2-pyridylthio)-1-indanone ethylene ketal from Step 7, (800 mg, 2.19 mmol), Fe powder (1.0 g, 17.8 mmol) and NH_4Cl (100 mg, 1.87 mmol) in 30 mL of EtOH:H_2O (2:1) was refluxed for 1 h. The hot mixture was filtered through celite. The solvent was evaporated *in vacuo*. The residue was diluted with H_2O and

extracted with EtOAc . Alternatively, the residue could be washed with H_2O, dried under vacuum to give the title compound (620 mg, quantitative) as a solid.
^1H NMR ($CDCl_3$): δ 8.36 (s, 1H), 7.95 (s, 1H), 7.40 (d, 1H), 7.78 (s, 1H), 7.76 (d, 1H), 3.05 (t, 2H), 2.64 (t, 2H).

Step 9: 5-Bis(methanesulfonyl)amino-6-(5-chloro-2-pyridyl-thio)-1-indanone

A mixture of 5-amino-6-(5-chloro-2-pyridylthio)-1-indanone from Step 8, (620 mg, 2.13 mmol), Et_3N (1.0 mL, 7.2 mmol) and MsCl (500 µL, 6.5 mmol) in CH_2Cl_2 (20 mL) was stirred at 0°C for 1 h. The mixture was then washed with saturated aqueous $NaHCO_3$, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Chromatography over silica gel and elution with hexanes:EtOAc (1:1) yielded the title compound (800 mg, 84%) as a foam.
^1H NMR ($CDCl_3$): δ 8.35 (s, 1H), 7.92 (s, 1H), 7.58 (s, 1H), 7.52 (d, 1H), 7.16 (d, 1H), 3.48 (s, 6H), 3.22 (t, 2H), 2.76 (t, 2H)

Step 10: 5-Methanesulfonamido-6-(5-chloro-2-pyridylthio)-1-indanone

To a solution of 5-bis(methanesulfonyl)amino-6-(5-chloro-2-pyridylthio)-1-indanone from Step 9, (800 mg, 1.79 mmol), in 30 mL of MeOH:THF (2:1) was added 1 M aqueous NaOH (10 mL, 10 mmol). The mixture was stirred at r.t. for 1 h and then quenched with HOAc (1 mL). Volatile solvents were evaporated *in vacuo*. The residue was diluted with H_2O, extracted with EtOAc. The EtOAc extract was washed with brine, dried over anhydrous $MgSO_4$ and concentrated. Chromatography over silica gel and elution with hexanes:EtOAc (1:2) gave the title compound (410 mg, 62%) as a pale yellow solid. Recrystallization from EtOH-EtOAc yielded the pure compound as needles.
^1H NMR ($CDCl_3$): δ 8.38 (s, 1H), 8.30 (s, 1H), 8.02 (s, 1H), 7.85 (s, 1H), 7.55 (d, 1H), 7.12 (d, 1H), 3.18 (t, 2H), 3.06 (s, 3H), 2.72 (t, 2H).

Examples 2 to 6 in Table I were prepared in the same manner as Example 1, Steps 7 to 10 by using 5-nitro-6-bromo-1-indanone ethylene ketal and the appropriate 2-mercaptopyridine. In the same way, Examples 7 to 17 can also be prepared.

PREPARATION OF STARTING MATERIALS

Preparation of 2-mercaptopyridines

5-Chloro-2-mercaptopyridine

Step 1: 5-Chloro-2-ethylthiopyridine

To a solution of 2,5-dichloropyridine (15.0 g, 0.10 mol) and ethanethiol (7.2 g, 0.12 mol) in DMF (100 mL) was added a solution of 10 M aqueous NaOH (22 mL, 0.22 mol) at r.t. The mixture was stirred at r.t. for 1 h and then heated at 100°C for 1 h. After cooling, the mixture was diluted with H_2O and extracted with Et_2O (2x). The combined ethereal extracts were washed with H_2O (3x), dried over anhydrous $MgSO_4$ and concentrated *in vacuo* to give the crude title compound (17.5 g, quantitative) as an oil.
^1H NMR ($CDCl_3$): δ 8.36 (s, 1H), 7.42 (d, 1H), 7.06 (d, 1H), 3.12 (q, 2H), 1.32 (t, 3H).

Step 2: 5-Chloro-2-ethanesulfinylpyridine

To a solution of 5-chloro-2-ethylthiopyridine from Step 1, (17.5 g, 0.10 mol) in chloroform (300 mL) at -78°C was added solid MCPBA (80-85% pure, 20.0 g, 0.092 mol) at -78°C. The mixture was allowed to slowly warm to 0°C over a period of 1-2 h, then washed with 1 M aqueous NaOH (2 x 200 mL) and H_2O. The organic solution was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*.
Chromatography over silica gel and elution with EtOAc afforded the title compound as an oil (16.8 g, 89%) which solidified on standing at r.t..
^1H NMR ($CDCl_3$): δ 8.55 (s, H), 7.90 (m, 2H), 3.15 (m, 1H), 2.88 (m, 1H), 1.18 (t, 3H).

Step 3: 5-Chloro-2-mercaptopyridine

A mixture of 5-chloro-2-ethanesulfinylpyridine from Step 2 (16.5 g, 0.087 mol) and TFAA (75 mL) was stirred at 0°C for 30 min, then refluxed for 1-2 h. Volatile materials were evaporated *in vacuo* to give the crude Pummerer rearrangement product in quantitative yield.

An aqueous methanolic NaOH solution was prepared from MeOH (300 mL) and 4 M aqueous NaOH (100 mL), cooled to 0°C and added to the above crude Pummer rearrangement product. The mixture was stirred at 0°C for 30 min. Volatile solvent was removed *in vacuo* and 1 M aqueous NaOH (200 mL) was added. The aqueous solution was washed with CH_2Cl_2 (2x), then acidified with HOAc. The yellow precipitate formed was collected, washed with H_2O and dried under vacuum at r.t. to give the title compound (5.5 g, 43%) as a yellow powder.

^1H NMR ($CDCl_3$): δ 7.65 (s, 1H), 7.42 (d, 1H), 7.30 (d, 1H).

The following 2-mercaptopyridines were also prepared according to the sequence shown in Method A as for 5-chloro-2-mercaptopyridine.

5-Bromo-2-mercaptopyridine

^1H NMR (Acetone-d_6): δ 7.85 (s, 1H), 7.42 (d, 1H), 7.25 (d, 1H).

3-Chloro-2-mercaptopyridine

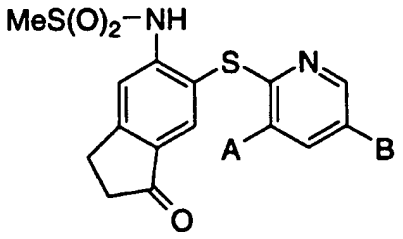
^1H NMR ($CDCl_3$): δ 7.70 (d, 1H), 7.60 (d, 1H), 6.75 (t, 1H).

3,5-Dichloro-2-mercaptopyridine

^1H NMR ($CDCl_3$): δ 8.02 (s, 1H), 7.64 (s, 1H).

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:-

1. A compound of formula I:



I

wherein:

A and B are independently:

(a) hydrogen,
(b) F, Cl, Br, or I,
(c) methyl or ethyl,
(d) ethenyl or ethynyl,
(e) OCH_3 or OCF_3,
(f) SCH_3 or SCF_3,
(g) CN, or
(h) N_3.

2. A compound according to Claim 1 wherein

A is

(a) hydrogen,
(b) F, Cl, Br,
(c) methyl or ethyl,
(d) ethenyl or ethynyl,
(e) OCH_3,
(f) SCH_3,
(g) CN;

B is

(a) hydrogen,

(b) F, Cl, Br,
(c) methyl,
(d) ethenyl or ethynyl,
(e) OCH_3,
(f) SCH_3, or
(g) CN

but A and B are not simultaneously hydrogen.

3. A compound according to Claim 2 wherein

A is
(a) hydrogen,
(b) F, Cl, Br,
(c) methyl,

B is
(a) hydrogen,
(b) F, Cl, Br, or
(c) methyl.

but A and B are not simultaneously hydrogen.

4. A compound selected from the group consisting of
(1) 5-Methanesulfonamido-6-(5-chloro-2-pyridylthio) -1-indanone;
(2) 5-Methanesulfonamido-6-(5-methyl-2-pyridylthio) -1-indanone;
(3) 5-Methanesulfonamido-6-(5-bromo-2-pyridylthio) -1-indanone;
(4) 5-Methanesulfonamido-6-(3-chloro-2-pyridylthio) -1-indanone;
(5) 5-Methanesulfonamido-6-(3,5-di-chloro-2-pyridylthio)-1-indanone;
(6) 5-Methanesulfonamido-6-(2-pyridylthio) -1-indanone;
(7) 5-Methanesulfonamido-6-(3-fluoro-2-pyridylthio) -1-indanone;

(8) 5-Methanesulfonamido-6-(5-fluoro-2-pyridylthio) -1-indanone;
(9) 5-Methanesulfonamido-6-(3,5-difluoro-2-pyridylthio) -1-indanone;
(10) 5-Methanesulfonamido-6-(5-chloro-3-fluoro-2-pyridylthio)-1-indanone;
(11) 5-Methanesulfonamido-6-(3-fluoro-5-bromo-2-pyridylthio)-1-indanone
(12) 5-Methanesulfonamide-6-(3-chloro-5-fluoro-2-pyridylthio)-1-indanone;
(13) 5-Methanesulfonamido-6-(5-methoxy-2-pyridylthio) -1-indanone;
(14) 5-Methanesulfonamido-6-(5-methylthio-2-pyridylthio)-1-indanone;
(15) 5-Methanesulfonamido-6-(5-ethenyl-2-pyridylthio)-1-indanone;
(16) 5-Methanesulfonamido-6-(5-ethynyl-2-pyridylthio)-1-indanone; and
(17) 5-Methanesulfonamido-6-(5-cyano-2-pyridylthio)-1-indanone.

5. A compound according to Claim 1 wherein

A is
- (a) F, Cl, Br,
- (b) methyl or ethyl,
- (c) ethenyl or ethynyl,
- (d) OCH_3,
- (e) SCH_3,
- (f) CN;

B is
- (a) F, Cl, Br,
- (b) methyl,
- (c) ethenyl or ethynyl,
- (d) OCH_3,

(e) SCH_3, or
(f) CN.

6. A compound according to Claim 5 wherein
A is
(a) F, Cl, Br,
(b) methyl,
B is
(a) F, Cl, Br, or
(b) methyl.

7. A pharmaceutical composition for treating an inflammatory disease susceptable to treatment with an non-steroidal anti-inflammatory agent comprising:
a non-toxic therapeutically effective amount of a compound according to Claim 1,2,3,4,5 or 6 and a pharmaceutically acceptable carrier.

8. A pharmaceutical composition for treating cyclooxygenase mediated diseases advantageously treated by an active agent that selectively inhibits COX-2 in preference to COX-1 comprising:
a non-toxic therapeutically effective amount of a compound according to Claim 1,2,3,4,5 or 6 and a pharmaceutically acceptable carrier.

9. A method of treating an inflammatory disease susceptable to treatment with an non-steroidal anti-inflammatory agent comprising:
administration to a patient in need of such treatment of a non-toxic therapeutically effective amount of a compound according to Claim 1 and a pharmaceutically acceptable carrier.

10. A m thod of treating cyclooxygenase m diated diseases advantageously treated by an active agent that selectively inhibits COX-2 in preference to COX-1 comprising:
administration to a patient in need of such treatment of a non-toxic therapeutically effective amount of a compound according to Claim 1.

11. A compound of Claim 1, 2, 3, 4, 5 or 6 for use in treating an inflammatory disease susceptible to treatment with a non-steroidal anti-inflammatory agent or for treating cyclooxygenase mediated diseases advantageously treated by an active agent that selectively inhibits COX-2 in preference to COX-1.

12. Use of a compound of Claim 1, 2, 3, 4, 5 or 6 in the manufacture of a medicament for treatment of inflammatory disease susceptible to treatment with a non-steroidal anti-inflammatory agent or for treating cyclooxygenase mediated diseases advantageously treated by an active agent that selectively inhibits COX-2 in preference to COX-1.

13. An anti-inflammatory pharmaceutical composition comprising an acceptable anti-inflammatory pharmaceutical amount of a compound of Claim 1,2, 3, 4, 5 or 6, in association with a pharmaceutically acceptable carrier.

14. A pharmaceutically acceptable acid addition salt of a compound of Claim 1, 2, 3, 4, 5 or 6.